PATENT
4957US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Houtzager et al.

Serial No.: 09/882,621

Filed: 15 June 2001

For: Chimaeric Phages

Examiner: To be assigned

Group Art Unit: 1642

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AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above identified patent application as follows:

IN THE SPECIFICATION:

On page 34, lines 6-31of the originally filed application, please replace the first full paragraph as follows:

The following primers (Genset, France) were used in the generation of the different vectors and helper phage genomic constructs. Most restriction enzymes hardly or fail to digest DNA if their corresponding palindrome is near the end of the DNA. Therefore a stretch of 8 nucleotides was added to the 5' end of all of these primers in which this stretch is an A/T rich non-hybridizing 8-mer.

D3 primers

D3 BamHI Forward

5'-GGATCC TCTGGTTCCGGTGATTTGATTATG-3' SEQ ID NO:1

D3 BamHI Backward

5'-GGATCC AGCGGAGTGAGAATAGAAAGGAAC-3' SEQ ID NO:2

g3-minus primers

g3 minus HindIII Forward

5'-AAGCTT CTGCGTAATAAGGAGTCTTAATCATGC-3' SEQ ID NO:3

g3 minus HindIII Backward

5'-AAGCTT GTTGAAAATCTCCAAAAAAAAAGGC-3' SEQ ID NO:4

g3 ORF primers

g3 ORF NcoI Forward

5'-CCATGG CTGAAACTGTTGAAAGTTGTTTAGC-3' SEQ ID NO:5

g3 ORF XbaI Backward

5'-TCTAGA TTAAGACTCCTTATTACGCAGTATG-3' SEQ ID NO:6

On page 40, line 30 through page 41, line 28 of the originally filed application, please replace the paragraph as follows:

The use of g3 minus HindIII Forward and g3 minus HindIII Backward primers and M13KO7 and VCSM13 as templates in a standard PCR reaction resulted in the formation of a PCR product that contained HindIII sites at both ends of the DNA. After separation, gel isolation and purification, digestion with HindIII (Roche Diagnostics) and re-purification of the DNA, the product was self-ligated under standard ligation conditions and electroporated into XL1Blue cells (Stratagene). The transformed cells were resuspended in 5 ml 2TY medium and cultured shaking at 37°C for 1 h. Kanamycin was added to an end-concentration of 50 μg/ml and the cells were allowed to grow

at the same conditions for another 5 h. The culture was centrifuged at 3000 rpm for 15 min and the supernatant passed through a 0.22 μM filter to remove bacteria. At the same time, a culture of exponentially growing XL-1 Blue bacteria was prepared. Fractions of the filtrate (50-1000 μl), containing phage particles, were added to 5 ml of XL-1 Blue bacteria and incubated at 37°C for 30 min without shaking. The culture was centrifuged again, the supernatant discarded and the cells were plated on 2X YT-K-T plates and transferred into an incubator at 37°C for overnight growth. Eight correct clones that lack the g3 ORF (checked through the BamHI site) and include the introduced HindIII site were isolated and used for g3-less helper phage production in the presence of the pBAD/gIII-g3 helper plasmid. Only two clones that were able to form phages in the presence of the helper plasmid were kept. From these clones a large quantity of DNA was isolated and stored for further experiments. The obtained g3-minus helper phage genome is depicted schematically in Figure 6A, while the correct sequence of this construct surrounding the HindIII and BsrI sites is depicted in Figure 6B as SEQ ID NO:7.

On page 41, line 33 through page 42, line 11 of the originally filed application, please replace the paragraph as follows:

The construction of helper phage genome that express only the D3 part of the g3 gene was comparable to the above described g3-less helper phages with the exception that the primers used were D3 BamHI Forward and D3 BamHI Backward primers in order to generate the new genome. All other procedures were the same as for the g3-less procedure, except for the use of BamHI instead of HindIII. In the end, the DNA of two correct clones was kept and stored at -20°C. The final construct of the helper phage genome that still expresses the part of the g3 gene that does not contribute to the infectiousness of the phage particle is depicted in Figure 7A, while the sequence surrounding the PCR product insert in the genome is depicted in Figure 7B as SEQ ID NO:8.

On page 47, line 34 through page 48, line 3, please replace the paragraph as follows:

Figure 6. (A) Schematic representation of the helper phage genome deleted for the open reading

frame (ORF) of the g3 gene. The arrow indicates the 3' end of the gene that remained after cloning procedures. (B) SEQ ID NO:7, which represents the sequence of the part of the helper phage genome that surrounds the position of the g3 deletion depicted in (A).

On page 48, lines 5-12, please replace the paragraph as follows:

Figure 7. (A) Schematic representation of the helper phage genome deleted for the part of the g3 gene that contributes to the infectivity of the phage. The arrow indicates the D3 part of the g3 gene that encodes the carboxy-terminal part of the g3 protein enabling the generation of stable, but essentially non-infectious helper phages. (B) SEQ ID NO:8, which represents the sequence of the nucleic acid that is shown in (A).

IN THE ABSTRACT:

Please insert the following page as the abstract.

REMARKS

Included herewith are: (1) A computer readable form (CRF) copy of the SEQUENCE LISTING for United States patent application serial no. 09/882,621 filed on June 15, 2001; (2) a paper copy of the SEQUENCE LISTING; and (3) a Statement per 37 C.F.R. §§ 1.821(f).

It is respectfully submitted that the specification, as originally filed, supports each of the amendments previously set forth, as well as the SEQUENCE LISTING included herein. The specification has been amended to insert the sequence identification numbers corresponding to the sequences listed in the originally filed specification. Support for the sequences identified by sequence identification numbers "1-6" can be found on page 34, lines 1-31 of the originally filed specification. Support for the sequence identified by sequence identification number "7" can be found on page 40, line 28 through page 41, line 28 and FIG. 6B of the originally filed specification. Support for the sequence identified by sequence identification number "8" can be found on page 41, line 30 through page 42, line 11 and FIG. 7B of the originally filed specification. Thus, it is respectfully submitted that this amendment includes no new matter.

Should the Office determine that additional issues remain, which might be resolved by a telephone conference, it is respectfully invited to contact applicants' undersigned attorney.

Respectfully Submitted,

Katherine Hamer

Katherine A. Hamer
Registration Number 47,628
Attorney for Applicants
TRASKBRITT, PC
P.O. Box 2550
Salt Lake City, Utah 84110
Telephone: (801) 532-1922

November 19, 2001

Enclosure: Version With Markings to Show Changes Made
CRF copy of the SEQUENCE LISTING
Paper copy of the SEQUENCE LISTING
Statement per 37 C.F.R. § 1.821(f)
Abstract

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

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